RESULTS

In the present investigation whole plants of *Asystasia travancorica* and *Sonerila tinnevelliensis* have been subjected to pharmaecochemical characterization, GC-MS analysis, *in vitro* antioxidant, anticancer, antidiabetic, hepatoprotective, antifertility antiinflammatory and immunomodulatory activities with a view to assess their pharmacological potentials.

**Analysis of the powdered drug**

**Ash and extractive values**

The results of the ash values and extractive values of the whole plants of *A. travancorica* and *S. tinnevelliensis* are depicted in Table 1a and Table 1b. The total ash content of the powdered plants of *A. travancorica* and *S. tinnevelliensis* are 8.44% and 9.32% respectively. The present study revealed that the extractive values of both the experimental plants are more for the water extract than the extractive values of other solvent extracts tested.

**Fluorescence analysis**

The results of fluorescence analysis of the whole plants of *A. travancorica* and *S. tinnevelliensis* are shown in Table 2 and Table 3. The whole plant powders of *A. travancorica* and *S. tinnevelliensis* as such, fluoresced green under day light and yellowish green under short UV light (254 nm). The powdered drug of *A. travancorica* fluoresced dark green under long light UV light (365 nm) whereas the powdered drug of *S. tinnevelliensis* fluoresced dark blue under long light UV light (365 nm). The powdered whole plant of *A. travancorica* emitted the characteristic fluorescent green colour when treated with 1N alcoholic NaOH, 50% sulphuric acid, 50% nitric acid concentrate nitric acid, 40% sodium hydroxide + 10% lead acetate, acetic acid and
petroleum ether. The powdered whole plant of *S. tinnevelliensis* emitted the characteristic fluorescent green colour when treated with 1N alcoholic NaOH, 50% nitric acid, concentrate nitric acid and ammonia.

**Preliminary Phytochemical Screening**

The distribution of different phytochemical constituents in petroleum ether, benzene, chloroform, methanol, ethanol and water extracts of whole plants of *A. travancorica* and *S. tinnevelliensis* were evaluated qualitatively and the results are presented in Table 4. The presence of phytocompounds such as alkaloids, anthraquinones, catehins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, sugars, glycosides and xanthoprotein have been confirmed in the methanol and ethanol extracts of the selected plants.

**HPTLC analysis**

The HPTLC profiles of alkaloid, flavoniod, glycoside, saponin and steroid in day light and UV lights at 254 nm and 366 nm wave lengths are given in Plates I - V. The Rf values, peak area and the type of substances assigned are given in Tables 5 - 9. The HPTLC densitograms of alkaloid, flavonoid, glycoside, saponin and steroid of both the investigated plants are given in Figures 1 - 10.

Both the plant extracts showed the presence of alkaloids, flavonoids, glycosides, saponins and steroids. The results revealed the presence of 3 types of alkaloids, 10 types of flavonoids, 9 types of glycosides, 14 types of saponins and 3 types of steroids in the whole plant of *A. travancorica* and 4 types of alkaloids, 8 types of flavonoids, 12 types of glycosides, 9 types of saponins and 4 types of steroids in the whole plant of *S. tinnevelliensis*. 
**GC-MS analysis**

The chemical composition of whole plants of *A. travancorica* and *S. tinnevelliensis* were analyzed by using GC-MS. The chromatograms of whole plants of *A. travancorica* and *S. tinnevelliensis* are shown in Figure 11 and Figure 12 respectively. Mass spectra were used to identify the structure of the detected compounds by comparing them with those in NITS ver 2. (National Institute of Standards and Technology) library.

Ten compounds were detected in the whole plant ethanol extract of *A. travancorica* (Table - 10). The results revealed that, Levo-à-El emene (41.97%), Tetra hydrosipirilloxanthin (17.07%), Stigmasterol (12.25%), Phytol (8.23%), 2,6,10 – Dodecartrien-1-ol, 3,7,11-trimethyl- [trans-farnesol] (7.03%), Ethyl iso-allocholate (4.62%) and Heptadecane 9-hexyl (3.21%) were found as the major components in the ethanol extract of *A. travancorica*.

Eight compounds were detected in the whole plant ethanol extract of *S. tinnevelliensis* (Table - 11). Tetra hydrosipirilloxanthin (18.50%), Ethyl iso-allochoate (18.27%), Linolelaidic acid, methyl ester (6.09%), Diisooctyl phthalate (6.09%), Stigma sterol (5.39%) and Heptadecane, 9-hexyl (5.39%) were found as a major components in the ethanol extract of *S. tinnevelliensis*. The mass spectra of some of the detected and identified compounds in the whole plants of *A. travancorica* and *S. tinnevelliensis* are presented in Figures 13 and 14 respectively.

**Total phenolic content and flavonoid content**

The total phenolic content and total flavonoid content of the whole plant methanol extract of *A. travancorica* are found to be 0.54g 100g⁻¹ and 0.52g 100g⁻¹ and that of *S. tinnevelliensis* are found to be 0.32g 100g⁻¹ and 0.51g 100g⁻¹ respectively.
Pharmacological studies

Antioxidant Activity

**DPPH radical scavenging activity**

DPPH radical scavenging activity of the whole plant petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plants of *A. travancorica* and *S. tinnevelliensis* are shown in Figures 15 and 16. The scavenging effect of all the extracts and also the standard increases with the increase in the concentration. Among the solvents tested, whole plant ethanol extract of *A. travancorica* exhibited DPPH radical scavenging activity of 96.36%, the maximum at 800 µg/ml concentration. The whole plant methanol extract of *S. tinnevelliensis* exhibited DPPH radical scavenging activity of 96.11%, the maximum at 800 µg/ml concentration.

**Hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity of various extracts such as petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of whole plants of *A. travancorica* and *S. tinnevelliensis* are shown in Figures 17 and 18. Among the solvents tested, the whole plant petroleum ether extract of *A. travancorica* exhibited 91.13% hydroxyl radical scavenging activity, the highest scavenging activity at 800 µg/ml concentration. The whole plant methanol extract of *S. tinnevelliensis* possessed 93.16% hydroxyl radical scavenging activity, a very potent activity at 800 µg/ml concentration.

**Superoxide radical scavenging activity**

All the extracts of whole plants of *A. travancorica* and *S. tinnevelliensis* were subjected to be superoxide scavenging assay and the results are shown in Figures 19 and 20. The results showed that the whole plant methanol extracts of *A. travancorica* and *S. tinnevelliensis* exhibited the maximum superoxide scavenging activity of
101.56% and 98.13%, at 800µg/ml concentration respectively. This scavenging activity of the investigated plants was more or less equal to that of ascorbic acid, the standard (98.86%).

**ABTS radical scavenging activity**

All the extracts of the whole plants of *A. travancorica* and *S. tinnevelliensis* were subjected to be ABTS radical cation scavenging activity and the results are shown in Figures 21 and 22. The whole plant methanol extract of *A. travancorica* exhibited ABTS radical cation scavenging activity of 89.84% at 800µg/ml concentration, the maximum ABTS radical cation scavenging activity, whereas the whole plant ethanol extract of *S. tinnevelliensis* exhibited a potent ABTS radical cation scavenging activity of 88.27% at 800 µg/ml concentration.

**Reducing power**

Figures 23 and 24 showed a comparison between the reducing abilities of different solvent extracts of the whole plants of *A. travancorica* and *S. tinnevelliensis* and ascorbic acid, the standard. Absorbance of the solution was increased with the concentration. Higher absorbance indicated the higher reducing power. Among the solvents tested, the whole plant ethanol extracts of both the experimental plants exhibited higher reducing activity.

**IC₅₀ values**

IC₅₀ values of different solvent extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) of *A. travancorica* whole plant and the standard ascorbic acid tested for DPPH radical scavenging activity were found to be 18.41µg/mL, 16.84µg/mL, 17.64µg/mL, 19.87µg/mL, 20.13µg/mL and 20.94µg/mL respectively. IC₅₀ values of the above solvent extracts of *A. travancorica* whole plant and the standard ascorbic acid tested for hydroxyl radical scavenging activity were found to be
respectively. IC<sub>50</sub> values of the different solvent extracts of <i>A. travancorica</i> whole plant and the standard ascorbic acid tested for super oxide dismutase assay were found to be 20.33μg/mL, 19.28μg/mL, 18.94μg/mL, 24.97μg/mL, 21.56μg/mL and 22.08μg/mL respectively. IC<sub>50</sub> values of the different solvent extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) of <i>A. travancorica</i> whole plant and the standard trolox tested for ABTS assay were found to be 18.09μg/mL, 17.42μg/mL, 17.94μg/mL, 24.97μg/mL, 21.56μg/mL and 21.37μg/mL respectively (Table 12).

IC<sub>50</sub> values of different solvent extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) of <i>S. tinneveliensis</i> whole plant and the standard ascorbic acid tested for DPPH radical scavenging activity were found to be 19.35μg/mL, 17.82μg/mL, 18.83μg/mL, 20.21μg/mL, 19.88μg/mL and 20.94μg/mL respectively. IC<sub>50</sub> values of the above solvent extracts of <i>S. tinneveliensis</i> whole plant and the standard ascorbic acid tested for hydroxyl radical scavenging activity were found to be 18.06μg/mL, 19.24μg/mL, 17.04μg/mL, 19.46μg/mL, 17.41μg/mL and 21.54μg/mL respectively. IC<sub>50</sub> values of the different solvent extracts of <i>S. tinneveliensis</i> whole plant and the standard ascorbic acid tested for super oxide dismutase assay were found to be 20.18μg/mL, 17.16μg/mL, 18.94μg/mL, 22.81μg/mL, 21.40μg/mL and 22.08μg/mL respectively. IC<sub>50</sub> values of the different solvent extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) of <i>S. tinneveliensis</i> whole plant and the standard trolox tested for ABTS assay were found to be 17.84μg/mL, 17.28μg/mL, 18.14μg/mL, 19.18μg/mL, 20.51μg/mL and 21.37μg/mL respectively (Table 13).

**Anticancer activity**

Antitumor activity of the whole plant ethanol extracts of <i>A. travancorica</i> and <i>S. tinneveliensis</i> was assessed by comparing the parameters such as relative weight of
organs, solid tumour volume, viable and non-viable cell counts, mean survival time and % of increase in life span between the DAL tumour bearing control and drug treated DAL tumour bearing mice. The results are shown in Tables 14 to 17. The relative weight of organs, solid tumour volume and viable cell count were increased significantly in tumour induced control. In contrary, the mean survival time and non-viable cell count were decreased in DAL bearing control (Group II) mice. Administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at the dose of 200 mg/kg and 400 mg/kg body weight, significantly (*p*<0.05; *p*<0.01) decreased the tumour volume and viable cell count. Non-viable cell count was significantly (*p*<0.01) higher in animals treated with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* when compared with tumour induced (DAL) control animals. The mean survival time of the DAL tumour bearing mice was increased to 35.65±0.47 (% ILS =80.05), and 37.54±0.34 (% ILS = 89.59) with the administration of whole plant ethanol extract of *A. travancorica*, at the dose of 200 mg/kg and 400 mg/kg body weight respectively. Similarly, the mean survival time of the DAL tumour bearing mice was increased to 27.18±0.25 (% ILS = 37.27) and 34.61±0.15 (% ILS = 74.79) with the administration of whole plant ethanol extract of *S. tinnevelliensis*, at the dose of 200 mg/kg and 400 mg/kg body weight respectively. This antitumour activity of the ethanol extracts of both the investigated plants is comparable to the antitumour activity of the standard drug vincristin (mean survival time 34.90±0.55 and % ILS = 76.26 at the dose of 80 mg/kg body weight).

Haematological parameters such as Hb, RBC, WBC and differential count of tumour bearing control mice (Group II), on day 30, were found to be significantly altered (Table 17) when compared to normal control (Group I). There
was an increase in the total WBC with a reduction of Hb content of RBC. A modest change was noticed in total RBC. In differential count of WBC, the neutrophil and eosinophil counts were increased with a reduction in lymphocyte count. The administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* helped to recover these altered haematological parameters to near normal.

**Immunomodulatory and antitumor activity of *A. travancorica***

**Effect of *A. travancorica* whole plant extract on body weight and tumour weight of S180-bearing mice**

All the mice in drug treated tumour induced groups survived following the treatments with any one of the three doses (100 mg/kg, 200 mg/kg and 300 mg/kg body weight) of whole plant ethanol extract of *A. travancorica* (ATW) until they were sacrificed for analysis of immunological features. When the tumour masses were removed, the mice treated with ATW had no significant change in body and tumour weight (Table 18) but showed lower tumour growth to certain extent when compared with S180 bearing control group. The inhibition percentage was 21.89%, 37.30% and 52.53% respectively, when the concentration of ATW extract was 100 mg/kg, 200 mg/kg and 400 mg/kg body weight. Higher concentrations appeared to show more effective inhibition. Effect of ATW on the organ weight is given in Table 18. There was an increase in the weight of spleen, thymus and liver in the S180 bearing mice. With the administration of ATW extract weight of spleen, thymus and liver in tumour bearing mice decreased considerably. The weight of kidney and lungs were decreased in S180 bearing mice. Treatment with ATW extract was helped to increase the weight of kidney and liver.
Effect of ATW extract on immune functions of S180-bearing mice

Effect of ATW extract on humoral immune function

The effect of ATW extract on humoral immune function was estimated by measuring quantitative haemolysis of sheep red blood cells in vivo. A significant ($p<0.001$) reduction in the assay was induced in S180 bearing control group (Table 19). Treatment with all the three concentrations of ATW extract markedly increased the antibody secreted by spleen cells in mice. The effect of ATW extract, at 300 mg/kg body weight dose, was the best to restore humoral immunity in S180 bearing mice, close to Cisplantin a standard drug to treat S180, at 20 mg/kg body weight.

Effect of ATW extract on cellular immune function

The effect of ATW extract on cellular immune function was estimated by measuring lymphocyte proliferation and NK cell cytotoxicity in vivo. In this assay, spleen lymphocyte proliferation and NK activity were significantly decreased ($p<0.001$; $p<0.05$) in S180 bearing control group. ATW extract, at 100 mg/kg, 200 mg/kg and 300 mg/kg, remarkably demonstrated spleen lymphocyte proliferation stimulation and increased NK activity, even higher to the action of the standard drug Cisplantin at 20 mg/kg body weight (Table-19). Regulation of cellular immunity close to the normal level was observed in NK cell cytotoxicity assay with the administration of ATW extract at different doses.

Effect of ATW extract on nonspecific immune function

The effect of ATW extract on nonspecific immune function was estimated by measuring the phagocytic activity of peritoneal macrophages in vivo. Administration with different doses of ATW extract significantly ($p<0.05$) enhanced the phagocytic activity of peritoneal macrophages in S180 bearing mice (Table 19). The extract,
at 300 mg/kg body weight, showed the most effective activity which was close to that of the normal control.

**Effect of ATW extract on Solid Tumour Volume**

There was a significant reduction of tumour volume in ATW treated mice (Table 20). Tumour volume of S180-bearing control mice was 4.88 on 30\textsuperscript{th} day while that of animals treated with ATW, at 300 mg/kg body weight dose, was only 2.28 on the same day.

**Effect of ATW extract on the mean survival time, increase in life span, viable cell count and non viable cell count**

Life span of S180 tumour bearing mice treated with the ethanol extract of ATW was significantly \((p<0.01)\) increased (Table 21). Tumour bearing control animals survived only 16.88±0.015 days while the animals treated with the ethanol extract of ATW at 300 mg/kg body weight dose survived up to 26.74±0.18 days with an increase in life span of 58.41%. Treatment with the ethanol extract of ATW significantly \((p<0.01)\) reduced the packed cell volume and viable cell count in a dose dependent manner as compared to that of the S180 tumour bearing control group. Furthermore, nonviable cell count in ATW extract treated groups was increased in a dose dependent manner.

**Effect of ATW extract on haematological parameters**

As shown in Table 22, the haemoglobin content in S180 tumour bearing mice (7.34 g\%) was significantly \((p<0.01)\) decreased when compared to normal control mice (12.84g\%). While the haemoglobin content in S180 tumour bearing mice treated with the ethanol extracts of ATW, at the doses of 200 mg/kg and 300 mg/kg body weight, increased to 11.48g\% and 12.87g\% respectively.
Effect of ATW extract on the bone marrow cellularity and $\beta$-esterase activity

The effect of ethanol extract of ATW on the bone marrow cellularity and $\beta$-esterase positive cells are given in Table 23. Administration with the ethanol extract of ATW (300 mg/kg body weight) showed a significant ($p< 0.05$) enhancement in the bone marrow cellularity (21.84 x $10^6$ cells/femur) when compared to S180 tumour bearing control (13.84x$10^6$ cells/femur) animals. Moreover, treatment of S180 tumour bearing mice with the ethanol extract of ATW (300 mg/kg body weight) helped to increase significantly ($p< 0.01$) the number of $\beta$-esterase positive cells (1194.62 cells/4000 bone marrow cells) when compared to the S180 tumour bearing control mice (724.10 cells/4000 bone marrow cells).

Effect of ATW extract on the antibody titer

The enhancement of total antibody production by the S180 tumour bearing mice administrated with the ethanol extract of ATW is shown in Table 24. The maximum antibody titer value (242.36) was observed on 15$^{th}$ day in S180 tumour bearing animals treated with the ethanol extract of ATW at 300 mg/kg body weight dose. The S180 tumour bearing control animals showed the maximum antibody titer value of only 191.36 ± 36 on the 15$^{th}$ day.

Antidiabetic activity

The impact of repeated oral administration with the whole plant ethanol extracts of *A. travancorica*, *S. tinneveliensis* and glibenclamide, the standard antibiotic drug, on body weight of the normal and diabetic rats, are shown in Table 25. The fasting blood glucose (FBG) level remains more or less the same, in normal control mice, before and after the treatment with saline only. Whereas, diabetic control rats showed a gradual increase in fasting blood glucose level after 2 weeks of treatment with vehicle (saline only). Moreover, treatment with the whole
plant ethanol extract of *A. travancorica* at 400 mg/kg body weight, the most effective dose, decreases FBG significantly from 198.53 mg/dl to 98.24 mg/dl and treatment with the whole plant ethanol extract of *S. tinnevelliensis* at 400 mg/kg body weight, decreases FBG significantly from 216.90 mg/dl to 109.34 mg/dl respectively. This sharp fall in fasting blood glucose level was a clear evidence of significant antidiabetic effect of whole plants of *A. travancorica* and *S. tinnevelliensis*.

Table 26 shows the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin in normal, diabetic control and diabetic drug treated experimental rats. When compared to normal control rats (Group I), the alloxan induced diabetic control rats (Group II) showed a significant elevation in the levels of blood glucose, urea, creatinine and glycosylated haemoglobin, while a decrease in the level of plasma insulin was noticed in diabetic induced control rats. Administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at 400 mg/kg body weight dose (Group IV & VI), and glibenclamide, the standard antibiotic drug, (Group VII) tends to bring back the above parameters to normal significantly. The effect of the selected plant extracts, at the dose of 400 mg/kg body weight, was highly significant in restoring normalcy.

The levels of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP, in the serum of diabetic rats, are presented in Table 27. The levels of serum total protein, albumin, globulin and elevated levels of liver marker enzymes such as SGPT, SGOT and ALP were decreased in the diabetic control rats (Group II) when compared to normal control rats (Group I). After treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at 400 mg/kg body weight dose (Groups IV and VI) and glibenclamide (Group VII), the total protein, albumin, globulin and liver marker enzymes were brought back to near normal
levels.

Table 28 shows the levels of TC, TG, HDL–C, LDL–C, VLDL–C and PL in the serum of normal control, diabetic control and diabetic drug treated rats. The diabetic rats when compared to normal control rats had elevated levels of serum TC, TG, LDL–C, VLDL–C and PL and a decreased level of HDL–C. Administration with the whole plant of ethanol extracts of *A. travancorica* and *S. tinnevelliensis* and the standard antibiotic drug, glibenclamide, helped to decrease TC, TG, LDL, VLDL and PL levels and also helped to increase the HDL–C level significantly (*p*<0.01).

The activities of LPO, GPx, GSH, SOD and CAT in the serum, liver and kidney of alloxan induced diabetic rats are illustrated in Tables 29 - 31. In the present study, the alloxan induced diabetic rats showed an increased activity of LPO and decreased activities of SOD, CAT, GPx and GSH in the serum, liver and kidney. Treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* and glibenclamide showed a reversal of all these parameters to near normal levels.

**Hepatoprotective activity**

The effect of whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on the body weight of normal control, CCl$_4$ intoxicated control and drug treated rat groups are shown in Table 32. An increase in body weight was noticed in all the groups except the rats in Group II, the liver damaged control, when compared to normal control. The loss in body weight loss was higher in liver damaged control rats than in normal control. Table 33 shows the effect of whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on serum total protein, albumin, globulin, A/G ratio, serum transaminases and alkaline phosphatases in normal control and CCl$_4$ intoxicated rats. There was a significant (*p*<0.001) increase in serum GOT,
GPT and ALP levels in CCl₄ intoxicated control (Group II) when compared to the normal control (Group I). The total protein has decreased slightly in CCl₄ intoxicated control. Whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at a dose of 400 mg/kg body weight helped to decrease the elevated serum marker enzymes significantly. Treatment with the standard drug silymarin and experimental plant extracts reversed the altered total protein and albumin to almost normal level. The effect of whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on total bilirubin, conjugated and unconjugated bilirubin and γ-glutamyl transferase are shown in Table 34. There was a significant (*p* < 0.001; *p* < 0.05) elevation of total bilirubin, conjugated and unconjugated bilirubin and γ-glutamyl transferase (GGT) or Gamma glutamyl transpeptidase (GGTP) in the serum of CCl₄ intoxicated control rats (Group II) as compared to normal control (Group I). The whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at the dose of 400 mg/kg body weight, reduced the elevated levels of total bilirubin, conjugated and unconjugated bilirubin in plant drug treated rats (Group IV & VI). Decrease in the concentration of total bilirubin, conjugated bilirubin, unconjugated bilirubin and γ-glutamyl transferase was found to be greater in Group IV rats treated with the whole plant ethanol extract of *A. travancorica* followed by Group VI rats treated with the ethanol extract of *S. tinnevelliensis*.

The effect of whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on lipid peroxidation (LPO), glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activities are shown in Table 35. When compared to the normal control rats (Group I), the level of lipid peroxidation had increased significantly (*p* < 0.01) and the levels of glutathione peroxidase, glutathione reductase,
superoxide dismutase, catalase and reduced glutathione had decreased significantly $(p<0.01)$ in CCl$_4$ intoxicated control rats (Group II). Treatment with the whole plant of ethanol extracts of *A. travancorica* and *S. travancorica* decreased the elevated lipid peroxidation level significantly and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and reduced glutathione levels towards normal in a dose dependent manner. The results were well comparable with that of silymarin treated rats.

**Antifertility activity**

**Body weight and reproductive organ weight**

Administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* to rats significantly $(p < 0.05; p < 0.01)$ changed the body weight and also the libido of treated rats (Table 36). A significant $(p <0.05)$ decrease in the weight of testis and other accessory sex organs was observed in all the drug treated rat groups. Among the accessory sex organs, a significant weight reduction was noticed in the caput and caudal epididymis segment. Administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* decreased the weight of vas deferens (VD), seminal vesicle (SV) and prostrate.

**Sperm count and sperm motility**

Treatment of male rats with the whole plant ethanol extracts of the experimental plants significantly decreased the sperm motility and sperm density in caudal epididymis (Table 37). This impact on sperm motility and sperm density was sever in Group V rats treated with the whole plant ethanol extract of *A. travancorica* followed by Group III rats treated with the whole plant ethanol extract of *S. tinneveliensis*. The same trend was noticed in the caput epididymis sperm density as compared to control rats (Group I).
Sperm abnormality

Treatment of male rats with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* caused a drastic (*p* <0.05) sperm abnormality in caput and caudal regions (Table- 37). Among the two plants selected for the present study, whole plant extract of *A. travancorica* showed a significant and drastic abnormality in the sperm morphology. Further the head region of the sperms, in all the treated groups, was much affected than the tail region.

Serum biochemical profile

Serum protein, albumin, globulin, urea and creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) in the control and plant drug treated rats were depicted in Table 38. When compared to control rats a slight decrease in serum protein and an increase in the levels of urea and liver marker enzymes were observed in the experimental rats treated with the plant extracts.

Reproductive hormone profile

Serum testosterone level

Repeated treatment of male rats with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, for 14 days, caused a significant (*p* < 0.01) decrease in the serum testosterone level (Table 39).

Serum luteinizing hormone (LH) level

Treatment of male rats repeatedly with the whole plants ethanol extracts of *A. travancorica* and *S. tinnevelliensis* caused a dose related and statistically significant (*p* < 0.05; *p* <0.01) decrease in the serum LH level (Table 39).

Serum estrogen level

A dose dependent increase in the serum estrogen level was observed in male rats treated with the whole plant ethanol extracts of *A. travancorica* and
Treatment of male rats with 400 mg/kg body weight dose, for 14 days, caused a sharp rise in the serum estrogen level (Table 39). The results obtained confirmed that the whole plant ethanol extract of *A. travancorica* was more effective than that of whole plant ethanol extract of *S. tinnevelliensis* in enhancing the serum estrogen level.

**Serum follicle stimulating hormone (FSH) level**

Treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, for 14 days, helped to increase the serum FSH level, in male rats as compared to control rats (Table 39). This increase in the serum FSH level was statistically significant (*p* < 0.01) and dose dependent.

**Fertility test**

The results presented in Table 40 showed that intragastric administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* (400 mg/kg body weight dose), for 14 days to male rats, caused a significant reduction (*p* < 0.01; *p* < 0.001) in the number of females impregnated by treated male rats. When compared with female impregnated with untreated male rats the number of viable foetuses, found after cesarean sections, were significantly (*p* < 0.01) decreased in female rats impregnated by treated males. On other hand, the number of resorption sites was decreased in female impregnated by treated male rats as compared to controls.

**Antiinflammatory activity**

The antiinflammatory activities of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* are presented in Table 41. Both the plant extracts inhibited the paw oedema in rats induced by carrageenan significantly. The percentage of inhibition was dose dependent and the percentage of inhibition of
paw oedema, at 3rd h of induction, by the ethanol extract of *A. travancorica* was 80.83% and 82.56% respectively for 250 mg/kg body weight and 500 mg/kg body weight concentration. The percentage of inhibition of paw oedema by the ethanol extract of *S. tinnevaldiana* at 250 mg/kg body weight and 500 mg/kg body weight dose was 66.50%, 78.11% respectively. The inhibition percentage of paw oedema by the extracts of the selected plants was statistically significant and well comparable (82.71%) to that of indomethacin, the standard antiinflammatory drug, at 10 mg/kg body weight dose.

**Immunomodulatory activity:**

Immunomodulatory activities of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevaldiana* were evaluated in Swiss albino female mice, after treatment with two different doses (200 mg/kg and 400 mg/kg body weight) of whole plant ethanol extracts of *A. travancorica* and *S. tinnevaldiana* for 5 days. Immunomodulatory activities pertaining to body weight, relative weight of organs, delayed type hypersensitivity (DTH) and haemagglutination titre (HT) were studied in all the treated animal groups. Of the two experimental plants, *A. travancorica* was more effective in increasing body weight and also in increasing weight of organs such as spleen, liver and kidney (Table 42).

The DTH response was comparatively increased, in a dose dependent manner, in mice treated with the whole plants extracts of *A. travancorica* and *S. tinnevaldiana*, at 200 mg/kg and 400 mg/kg body weight doses when compared to control rats. The haemagglutinin titre (HT) also showed a significant increase (Table 43). There was a significant elevation in the levels SGOT, SGPT and ALP in rats treated with the plant extracts. The whole plant extracts of *A. travancorica* and *S. tinnevaldiana*, at 400 mg/kg body weight dose, showed a significant difference in some of the blood
parameters studied. Both the plant extracts, at 200 mg/kg and 400 mg/kg body weight doses, increased the WBC content in treated rat groups (Table 44).